

LESSON: Picking Up on Preservatives

Summary: Students read an article and analyze data about metabolites the body uses to get rid of parabens (a group of chemicals used as preservatives). Based on these data, students hypothesize about which metabolites may make the best biomarkers for determining paraben exposure.

Lesson Type: Data Analysis—Students read and interpret data from graphs or figures.

EHP Article: “Picking Up on Preservatives: New Biomarkers for Gauging Paraben Exposure”
EHP Student Edition, March 2007, p. A714
<http://www.ehponline.org/docs/2006/114-12/ss.html#pick>

Objectives: By the end of this lesson, students should be able to

1. identify features that make a good biomarker;
2. describe why scientists need a useful biomarker for parabens;
3. read a data table and extract important information; and
4. synthesize information from the lesson, data table, and article to generate a hypothesis.

Class Time: 40–50 minutes

Grade Level: 11–12

Subjects Addressed: Biology, Biochemistry, Molecular Biology, Environmental Health

► Prepping the Lesson (15 minutes)

INSTRUCTIONS:

1. Download the entire March 2007 *EHP Student Edition* at <http://www.ehponline.org/science-ed/>, or download just the article “Picking Up on Preservatives: New Biomarkers for Gauging Paraben Exposure” at <http://www.ehponline.org/docs/2006/114-12/ss.html#pick>.
2. Review the Background Information, Instructions, and Student Instructions.
3. Make copies of the Student Instructions and article.

MATERIALS (per student):

- 1 copy of the March 2007 *EHP Student Edition*, or 1 copy of “Picking Up on Preservatives: New Biomarkers for Gauging Paraben Exposure,” preferably in color
- 1 copy of the Student Instructions

VOCABULARY:

- anabolism
- antimicrobial
- bioactivity
- biomarker
- catabolism
- conjugate
- estrogenic
- hydrolysis
- metabolism
- metabolite
- paraben



BACKGROUND INFORMATION:

This lesson complements the March 2007 *EHP Student Edition* lesson, "What Are Biomarkers?" Although the lessons can be implemented independently, it may be helpful for students to have some familiarity with biomarkers prior to doing this lesson.

In short, biomarkers are specific physical traits or biologically produced chemicals connected with a disease or health condition. For example, physicians can measure elevated blood glucose levels as an indicator for diabetes or the potential for a person to develop diabetes. It has only been in the last 15 to 20 years that biomarkers have started being identified to elucidate exposures to environmental agents and the development of a disease potentially related to that exposure.

Research published in the December 2006 issue of *EHP* (Ye et al.) shows the potential of using specific conjugated metabolites of various forms of paraben as biomarkers for paraben preservatives found in foods and cosmetics. Researchers found that the body excretes, through urine, glucuronidated and sulfated forms of parabens, as well as small amounts of unchanged forms (conjugates) of the parabens.

RESOURCES:

Environmental Health Perspectives, Environews by Topic page, <http://ehp.niehs.nih.gov/>. Choose Chemical Exposures, Standards

Environmental Health Perspectives, Signs of the times: biomarkers in perspective, <http://www.ehponline.org/docs/2006/114-12/focus-abs.html>

Darbre PD, Aljarrah A, Miller WR, Coldham NG, Sauer MJ, Pope GS. 2004. Concentrations of parabens in human breast tumours. *J Appl Toxicol* 24:5–13.

van den Berg M, Sanderson T, Kurihara N, Katayama A. 2003. Role of metabolism in the endocrine-disrupting effects of chemicals in aquatic and terrestrial systems. *Pure Appl Chem* 75:1917–1932.

Wikipedia, Conjugate, <http://en.wikipedia.org/wiki/Conjugate>

Wikipedia, Metabolism, <http://en.wikipedia.org/wiki/Metabolism>

Ye X, Bishop AM, Reidy JA, Needham LL, Calafat AM. 2006. Parabens as urinary biomarkers of exposure in humans. *Environ Health Perspect* 114:1843–1846; <http://www.ehponline.org/members/2006/9413/9413.html>

► Implementing the Lesson**INSTRUCTIONS:**

1. Provide a brief introduction to biomarkers as needed (refer to the background section and the March 2007 *EHP Student Edition* article "Signs of the Times: Biomarkers in Perspective" at <http://www.ehponline.org/docs/2006/114-12/focus-abs.html> for information). Also, introduce or review the concept of metabolites, and specifically conjugation, as a way for the body to get rid of certain chemicals. Tell students they will analyze data and synthesize information to form a hypothesis about the potential health risk from exposure to methyl and *n*-propyl parabens.
2. Distribute the Student Instructions and the article.
3. Discuss the answers with the students, as needed, to advance their skills and understanding.

NOTES & HELPFUL HINTS:

1. This lesson complements the March 2007 *EHP Student Edition* lesson "What Are Biomarkers?" The lesson can be found at <http://www.ehponline.org/science-ed/> under the March 2007 listing.
2. Minimal background knowledge is needed to do this lesson, but students do need to be able to extract key information without getting too distracted by the lingo. Although the lesson is recommended for more advanced students, the skills developed in the lesson are beneficial to all students. Less advanced students will simply need more guidance and support from the teacher.

► Aligning with Standards**SKILLS USED OR DEVELOPED:**

- Classification
- Communication (note-taking, oral, written—including summarization)
- Comprehension (listening, reading)
- Critical thinking and response
- Tables and figures (reading)



SPECIFIC CONTENT ADDRESSED:

- Biomarkers
- Conjugates
- Median
- Metabolism
- Metabolites
- Parabens
- Preservatives
- Range

NATIONAL SCIENCE EDUCATION STANDARDS MET:**Science Content Standards****Unifying Concepts and Processes Standard**

- Systems, order, and organization
- Evidence, models, and explanation
- Change, constancy, and measurement
- Evolution and equilibrium
- Form and function

Science as Inquiry Standard

- Understanding about scientific inquiry

Physical Science Standard

- Chemical reactions

Life Science Standard

- The cell
- Molecular basis of heredity
- Matter, energy, and organization in living systems

Science and Technology Standard

- Abilities of technical design
- Understanding about science and technology

Science in Personal and Social Perspectives Standard

- Personal and community health
- Environmental quality
- Natural and human-induced hazards
- Science and technology in local, national, and global challenge

► Assessing the Lesson

Step 1: a. In your own words, explain the purpose of biomarkers.

Scientists use biomarkers to identify whether an exposure has occurred, a disease/illness has progressed, or a treatment is working.

b. Why is *p*-hydroxybenzoic acid not necessarily a useful biomarker?

Look for a logical, accurate, and clearly written answer that addresses the fact that there are many different types of parabens that people are exposed to, and that *p*-hydroxybenzoic acid is a metabolite produced for all of the parabens. Thus, *p*-hydroxybenzoic acid does not allow a researcher to differentiate between exposures to different paraben types or amounts of the different types of parabens. We want to be able to differentiate between different types of parabens because they may produce different health effects (they have different levels of estrogenic action).



- c. Why do researchers want to have good biomarkers for parabens?

Look for a logical, accurate, and clearly written answer that addresses any of the following:

- Humans are exposed to different types of parabens from many different sources, and biomarkers will help us determine the amounts of different parabens that are absorbed by the body from this variety of sources.
- Biomarkers will help researchers understand how the body processes or gets rid of parabens.
- The biomarkers may help determine potential health risks from exposure to parabens.

- Step 2:** a. If you had to pick one form of the methyl paraben compound (free, glucuronide, or sulfate) as a biomarker, which would it be and why?

Students answers may vary; look for logical explanations for their answer. A more obvious answer would be to use the sulfate form because that appears to be the most common methyl paraben-specific metabolite. Thus, if someone were exposed to methyl paraben, you would most likely be able to identify that the person was indeed exposed. If you used the other forms, the exposure may be missed.

- b. Looking at both the methyl and *n*-propyl parabens, what appears to be the body's preferred conjugate form to get rid of parabens?

It appears that the body prefers to make sulfated conjugates.

- c. Research by other scientists shows that the unconjugated or free form of some parabens may be biologically active, potentially resulting in unwanted negative health effects. Research has also shown the presence of unconjugated or free forms of parabens in breast cancer tissue. Consider the information just provided and the information in the table. What would you hypothesize about the potential health risk from exposure to methyl and *n*-propyl parabens? Consider factors like metabolism of parabens, potential storage of parabens, and biological activity. Be sure to state any assumptions you are using in your answer.

Student answers may vary and take different positions. Students may provide a reasonable answer not included here. Make sure the answers are clearly communicated, logical, and sufficiently explained. Possible answers include:

- According to the table, the largest percentage of excreted parabens are conjugated, and only 2–5% are unconjugated or free. This may indicate that the majority of parabens introduced in the body are conjugated and excreted, therefore posing potentially little health risk. Assumptions in this hypothesis are: 1) the conjugates are not biologically active or harmful; 2) even though there is some evidence of retention of unconjugated parabens in human tissue, a very small percentage is retained in the tissue; and 3) a small amount of biologically active conjugate retained in human tissue is not harmful.
- According to the table, as much as 5% of the parabens excreted are the biologically active form. Research also shows that unconjugated parabens are being retained by human tissues. This may indicate that parabens have a potential health risk. Assumptions in this hypothesis are: 1) unconjugated paraben has a high biological activity when retained in tissue; 2) enough parabens are retained in tissues to cause a health problem; and 3) the 5% that is excreted may be only a small portion of what is retained by the body (we would need to know the level of initial exposure to determine how much is excreted or retained).

► Authors and Reviewers

Author: Stefani Hines, University of New Mexico, College of Pharmacy

Reviewers: Stephanie Bishop, Susan Booker, Erin E. Dooley, Laura Hemminger, and Joseph Tart

Give us your feedback! Send comments about this lesson to ehpscienceed@niehs.nih.gov.



STUDENT INSTRUCTIONS: Picking Up on Preservatives

Step 1: Read the article "Picking Up on Preservatives: New Biomarkers for Gauging Paraben Exposure." Then answer the following questions.

- a. In your own words, explain the purpose of biomarkers.

- b. Why is *p*-hydroxybenzoic acid not necessarily a useful biomarker?

- c. Why do researchers want to have good biomarkers for parabens?

Step 2: Most substances that enter the body are metabolized or modified by cells to either get energy by breaking apart a molecule (**catabolism**) or build new molecules for cell parts and functions (**anabolism**). Sometimes chemicals can pass through the body unchanged; other times, the body **conjugates** a chemical to get rid of it. There are different types of conjugation, or binding the unwanted chemical to an acid, to make the unwanted chemical water-soluble and easily excreted in the urine.

On the next page is a table from the actual scientific study on biomarkers for parabens described in the article "Picking Up on Preservatives: New Biomarkers for Gauging Paraben Exposure." Look at the table and answer the questions that follow.



Table 2. Urinary concentrations of the free, glucuronidated, and sulfated conjugates of methyl and *n*-propyl parabens in adults (*n* = 100).

Compound	Frequency of detection (%)	Median (ng/mL)	Range (ng/mL)	Percentage of total amount
Methyl paraben, free	75	0.8	< LOD–717	5
Methyl paraben, glucuronide	85	9.7	< LOD–1,670	28
Methyl paraben, sulfate	96	29.9	< LOD–1,300	67
<i>n</i> -Propyl paraben, free	37	< LOD	< LOD–95.0	2
<i>n</i> -Propyl paraben, glucuronide	64	3.2	< LOD–820	43
<i>n</i> -Propyl paraben, sulfate	83	5.2	< LOD–424	55

The LODs were 0.13 ng/mL (methyl paraben) and 0.18 ng/mL (*n*-propyl paraben). For the statistical calculations, concentrations < LOD were imputed a value of LOD divided by the square root of 2.

- If you had to pick one form of the methyl paraben compound (free, glucuronide, or sulfate) as a biomarker, which would it be and why?
- Looking at both the methyl and *n*-propyl parabens, what appears to be the body's preferred conjugate form to get rid of parabens?

- c. Research by other scientists shows that the unconjugated or free form of some parabens may be biologically active, potentially resulting in unwanted negative health effects. Research has also shown the presence of unconjugated or free forms of parabens in breast cancer tissue. Consider the information just provided and the information in the table. What would you hypothesize about the potential health risk from exposure to methyl and *n*-propyl parabens? Consider factors like metabolism of parabens, potential storage of parabens, and biological activity. Be sure to state any assumptions you are using in your answer.

